

PHENOTYPIC VARIABILITY AND SEED YIELD OF *JATROPHA CURCAS* L. INTRODUCED TO TUNISIA

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ABSTRACT

Eight accessions of *Jatropha curcas* (six American and two African) were sown in the south of Tunisia (Gabès) to study its agricultural properties and development under local semi-arid conditions. Traits observed included general aspects of plant development (plant height, canopy circumference, number of nodes and seed yield); leaf characteristics (petiole length, limb length, limb width and foliar surface); and seed traits (size and shape). Morphological analysis of seeds included seed image area, perimeter, length/width ratio, circularity index and J index. This index reflects the similarity of seed images with an ellipse showing differences in seed shape between accessions. *Jatropha curcas* genotypes introduced in Tunisia are polymorphic, their seed yield is low and variable between genotypes. Seed morphology analysis gave an indication in relation with the productivity in some of the accessions. The comparative agronomic studies are crucial before early selection.

Key words: diversity, morphology of *Jatropha curcas*, seed shape, seed yield, Tunisia.

RESUMEN

Se sembraron en el sur de Túnez (Gabès) semillas de ocho accesiones de *Jatropha curcas* (seis americanas y dos africanas) para estudiar sus propiedades agrícolas y desarrollo

en condiciones locales semiáridas. Los caracteres observados incluyen aspectos generales del desarrollo de planta (altura, circunferencia del follaje, número de nodos y producción de semillas); características de las hojas (longitud del peciolo, longitud del limbo, anchura del limbo y superficie foliar); y caracteres de la semilla (tamaño y forma). El análisis morfológico de las semillas incluyó el área de las imágenes, el perímetro, la relación longitud/anchura, el índice de circularidad y el índice J. Este índice refleja la semejanza de las imágenes de cada semilla con una elipse mostrando diferencias en la forma de las mismas entre accesiones. Los genotipos de *J. curcas* introducidos en Túnez son polimórficos, su producción de semilla es baja y variable entre genotipos. El análisis morfológico de las semillas da una idea en relación con la producción en algunas de las accesiones. Estudios agronómicos comparativos son cruciales antes de la selección.

Palabras clave: diversidad, forma de la semilla, morfología de *Jatropha curcas*, rendimiento, Túnez.

INTRODUCTION

Jatropha curcas L. (Physic nut) is a perennial, monoecious, deciduous, stem-succulent shrub belonging to the family Euphorbiaceae. It has a smooth bark, sturdy branches, and thick papery leaves (Fig. 1). The leaves are 8 to 18 cm wide, shiny and glabrous, with exiguous and pilose stipules. The petiole is 10-16 cm long. The inflorescence is monoecious. The fruit is a capsule, 3-4 cm long and 2.5-3.0 cm wide. The seeds, 1.5-2.0 cm long and 1.0-1.2 cm wide, are elliptical and black. Dormancy is induced by fluctuations in rainfall and temperature/light. The branches contain latex. It has well-developed roots; normally five roots are formed from seedlings, one central and four peripheral. A tap root is not usually formed by vegetative propagation (Heller, 1996; Ye et al., 2009).

The genus *Jatropha* includes 175 species in the world (Dehgan and Webster, 1976; Ye et al., 2009). The center of diversity of *Jatropha* is the Mesoamerican region (Mexico and Central America), which is illustrated by the fact that more than 100 out of 175 species of *Jatropha* are native to that region. There are 41 native species in Mexico, of which 31 are strictly endemic (Jiménez and Vega, 2011). Several species of *Jatropha* are native to South America (Ovando-Medina et al., 2011a). *Jatropha curcas* is native of Belize, Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua and Panama, and widely present throughout Central America, Africa and Asia, where it is grown as a hedge plant (Basha et al., 2009; Subramanyam et al., 2009; Ovando-Medina et al., 2011a).

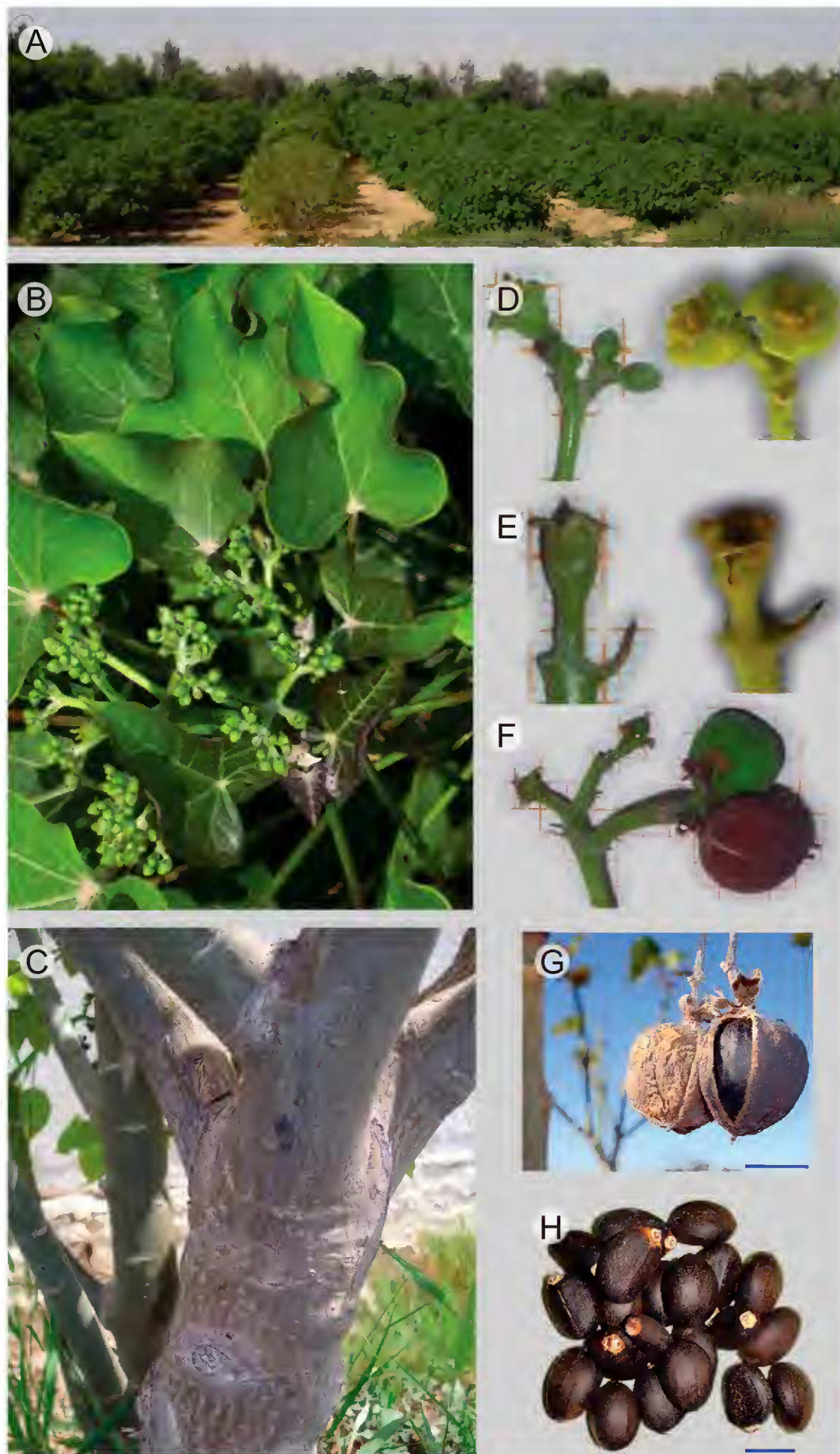


Fig. 1. A. View of experimental field. B to H. *Jatropha curcas* plants: B. leaves with flower buds; C. trunk with branches; D and E male and female flowers; F and G. fruits; H. seeds.

Jatropha curcas is a polymorph species with variations reported in tree architecture, plant height, number of fruits, seed dimensions (length, width, lateral diameter) and seed weight (Das et al., 2010; Rafii et al., 2012; Xu et al., 2012; Guan et al., 2013). With respect to molecular markers, the level of variation is different in localities. In America, where *J. curcas* is autochthonous, molecular analysis revealed high genetic variation (Ovando-Medina et al., 2011b; He, 2011; Brasileiro et al., 2013; Salvador-Figueroa et al., 2014). In contrast, assessment of *J. curcas* germplasm, using molecular markers in Asia and Africa, indicated low genotypic diversity and close clustering of accessions from both regions indicating a common ancestor (Basha and Sujatha, 2007; Singh et al., 2010; Zhang et al., 2011; Xu et al., 2012). This narrow level of variation has been attributed to the small number of introductions and their vegetative propagation (Khurana-Kaul et al., 2012).

Jatropha curcas has mainly evolved for xerophytic adaptation and is naturalized in the seasonally dry tropics, particularly in the southern hemisphere where there is no severe and prolonged frost (Xu et al., 2012). It grows well with 600 mm of rainfall per year or more. Deficit irrigation and salinity stress significantly reduced the growth and leaf development of greenhouse-grown *Jatropha* plants (Niu et al., 2012).

Conventional agriculture uses seeds and cuttings for its propagation, but *J. curcas* is amenable for *in vitro* propagation (Kumar et al., 2011). The planting of this species was motivated by the belief that it grows easily with minimal management requirements, but soil restoration and water conservation techniques improve the growth and survival rates on degraded soils (Kagamèbga et al., 2011; Iiyama et al., 2012). In plantation, spacing was the important factor along with the cutting management program for long time cultivation (Srinophakun et al., 2011). Thus, the productivity of *J. curcas* varies with genotypes, varieties and ecological conditions (Sosa-Segura et al., 2012).

Jatropha curcas has acquired global interest and significant economic importance for its seed oil which can be converted to biodiesel, and is emerging as an alternative to petro-diesel (Sosa-Segura et al., 2012; Guan et al., 2013). Seeds contain about 30-65% oil (Nzikou et al., 2009; Basha et al., 2009; He, 2011).

Data concerning seed yield in *Jatropha curcas* are heterogeneous. In Mexico it ranged from 0.2 to 12 tons per hectare, depending on production conditions (Francis et al., 2005) while in Thailand, one year old plants produced 318 g seeds \times plant⁻¹ (794 kg per hectare; Heller, 1996). In India, in a study involving sixteen genotypes, three year old plants produced from 34 to 196 g seeds \times plant⁻¹ (Das et al., 2010). Rao et al. (2008) reported yields of more than 2 kg of dry seed per five year old plant in

Central India. In Egyptian deserts, *Jatropha* trees, irrigated with primary treated municipal wastewater, produced 2 tons seed \times 4000 m² (Tewfik et al., 2012). In general, yield is related with the age of the plants and thus, studies on grain yield need to include plants of certain age.

Recently, eight *J. curcas* accessions were introduced to Tunisia with the aim of studying the possibility of *Jatropha* culture and exploitation. Experimental sites are installed from North to South to estimate differences between accessions or environments relative to their development and productivity. This study aims to determine the morphological variation between eight germplasm accessions of *J. curcas*, grown under the same environmental conditions in the experimental Dissa site in the region of Gabès (South of Tunisia), and to analyze diversity in plant development, seed morphology and seed yield. This work will contribute to identify agronomic traits of interest and to select germplasm accessions which are better adapted to local environmental conditions.

MATERIALS AND METHODS

Plant material

Eight *Jatropha curcas* accessions were introduced to Tunisia from four countries: Brazil, Suriname, Mozambique and Tanzania (Fig. 2).

Experimental site

This study was conducted at the Dissa experimental station (Gabès, Tunisia; 33°54' N and 10°02' E; Fig. 1A), in an area of 1000 m², at an altitude of approximately 44 meters. This region belongs to the lower semi-arid bioclimate; mean annual rainfall averages 130 mm, average annual Potential Evapo-Transpiration (PET) is about 1400 mm. The annual average temperature is 24.5 °C, maximum and minimum temperatures are 34 °C and 12.8 °C, respectively. The soil is calcimagnesian with 15-35% of gypsum (CaSO₄2H₂O) at depths of 50-100 cm and 15% of Calcium carbonate (CaCO₃). The soil texture was drained and sandy; 85% sand, 10% clay and 5% silt. PH is 7.7 and electric conductivity EC = 2.2 mS/cm.

Water of irrigation

Plants were irrigated with treated wastewater, characterized by pH = 6.9, EC = 4.670 mS/cm, COD (Chemical Oxygen Demand) = 168 mgO₂/l and BOD₅ (Biochemical Oxygen Demand) = 40 mg O₂/l. The rate of Cl⁻, Ca⁺⁺, Mg⁺⁺ and SO₄²⁻ of

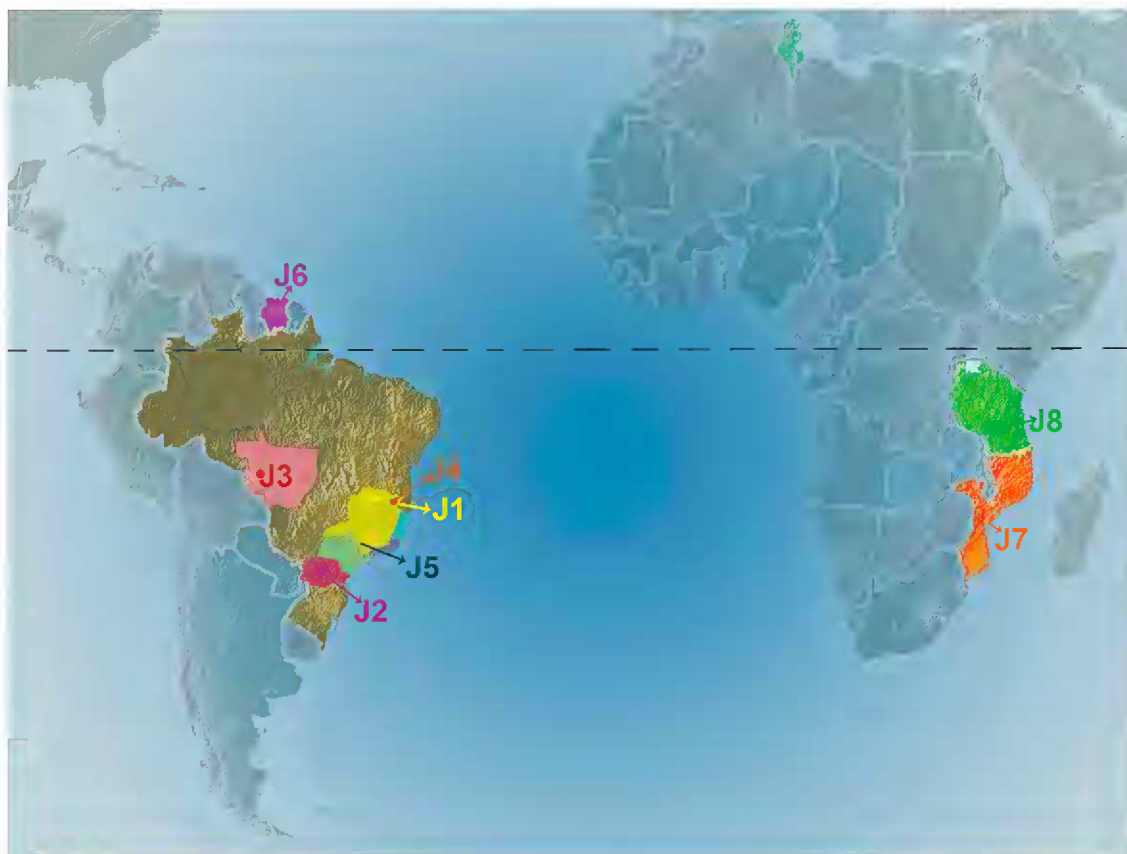


Fig. 2. Origin of the *Jatropha curcas* accessions used in the present study. The five Brazilian accessions (J1 to J5) proceed from: Norte de Mina (J1), Paranà (J2), Mato-Grosso (J3), Vale do Jequitinhonha (J4) and Regiao Sureste (J5). J6 is from Suriname, and J7 and J8 from Mozambique and Tanzania respectively.

treated wastewater is 39.4, 0.44, 0.32 and 450 mg \times l⁻¹ respectively. The quantity and frequency of irrigation were of 50 l \times week⁻¹ for a surface of 3 m².

Experimental protocol, development and yield of plants

Seedlings (25 per accession) were planted in March 2008. The experiment was conducted in a randomized block with 40 plants per plot, and 5 replicates. Each accession is represented by five plants in each plot. The spacing was 2 m x 3 m. Plant height (PH) was measured for each individual every year (2008-2013) and canopy circumference (CC) was measured in 2012. Mature fruits were harvested in the three periods of fruit maturity: August 2011, August and November 2012, and January and August 2013. Seeds obtained were weighed by electronic balance (SCALTEC SBA 41) for each plant (e = 0.1 g).

Morphological parameters

Petiole length (PL), limb length (LL) and limb width (LW) were measured by graduated ruler (e = 1 mm). Foliar surface (FS) was measured by a digital planim-

eter, with 0.1 cm² resolution and 0.2% precision. Measurements where mean values of five repetitions. Number of nodes (NN) was counted on five branches of 20 cm.

Seed morphology

Seeds were placed in graph paper over a flat surface, oriented with their micropile to the right side and photographed with a Nikon D60 camera adapted in a tripod. Forty seeds per accession where used to a total of 320 images. Five measurements in relation with morphology where obtained from each image: area, perimeter, length/width, circularity index and J index.

Circularity index (Schwartz, 1980) is a measure of the similarity of a plane figure to a circle given by

$$I=4\pi\frac{\text{area}}{(\text{perimeter})^2}$$

it ranges from 0 to 1 giving the value of 1 for circles.

An ellipse can be defined as the locus of all points that satisfy the equation

$$\frac{a^2}{x^2} + \frac{b^2}{y^2} = 1$$

where (x, y) are the coordinates of any point on the ellipse, and (a, b) are the radius on the x and y axes respectively.

Seed images of *Jatropha curcas* were adjusted to an ellipse whose relation between the major and minor axes (a and b) equals the Golden Ratio (1.618). The J index was defined previously for quantification of *Arabidopsis* seed shape in comparison to a cardioid (Cervantes et al., 2010), and is used here to measure the adjustment of seed shape to the ellipse. To obtain the J index, composed images containing an ellipse and the seed (Fig. 3) were elaborated for each seed with the software image Photoshop CS5.1 (Adobe). Area quantification was done with Image J (Java Image Processing Program).

Quantification of the adjustment was done in each seed as a proportion between the areas in two regions: the common region in the ellipse and the seed image (area C), and the sum of total regions of both images, the seed and the ellipse (Fig. 3). The index of adjustment (J index) is defined by:

$$J=\frac{\text{area}(C)}{\text{area}(C)+\text{area}(D)}$$

where area (C) represents the common region and area (D) the regions not shared. Note that J index ranges between 0 and 1, and decreases when the size of the non-

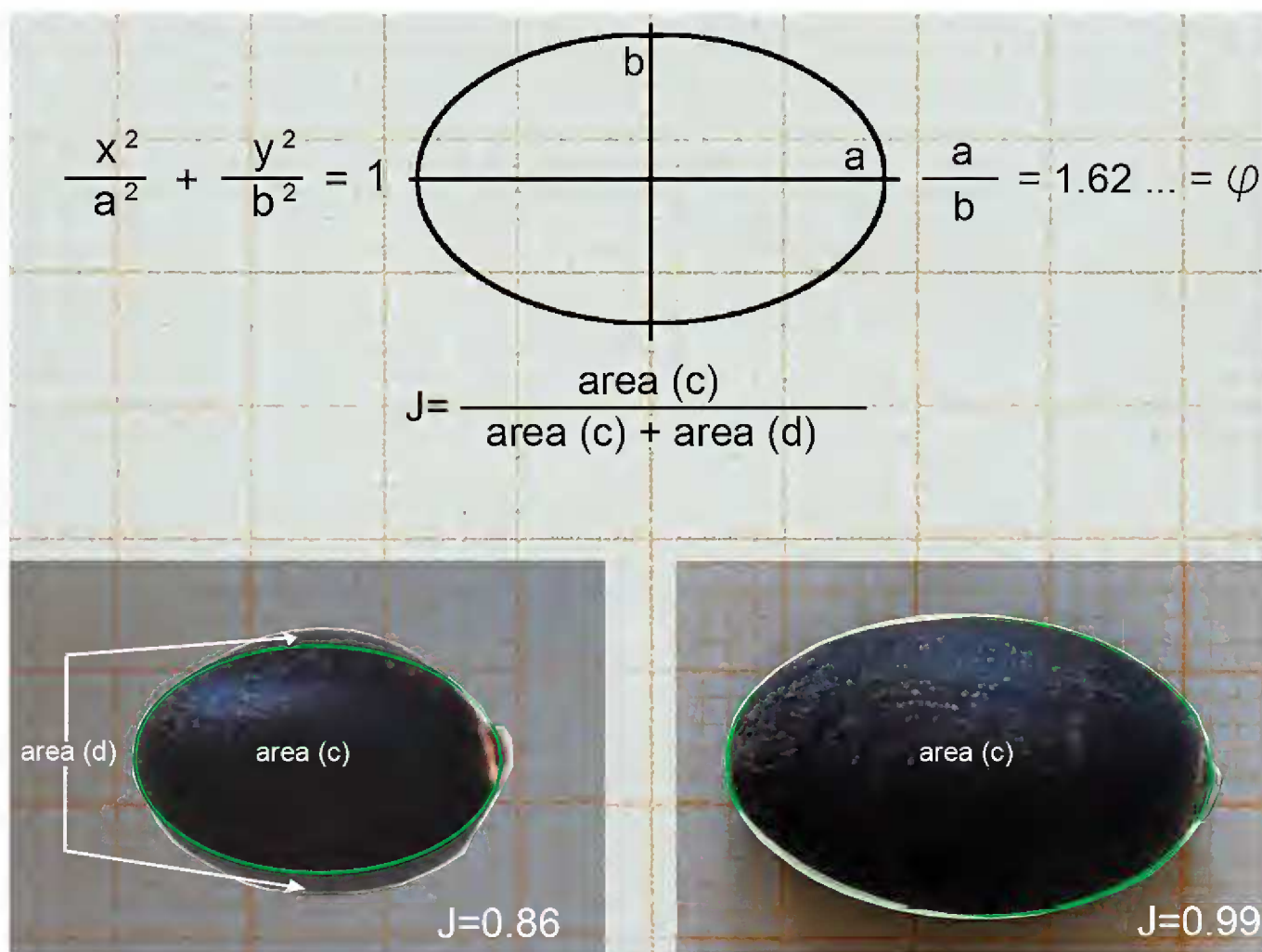


Fig. 3. Adjustment of seed shape to an ellipse whose relation between major and minor axis (a and b) equals the golden Ratio. Index J is the ratio between the area shared by the seed image and the ellipse and the sum of shared and not shared area.

shared region grows. It equals 1 when ellipse and seed image areas coincide, i.e., area (D) is zero. J Index was calculated for a total of 320 seeds (40 seeds per each of eight accessions).

Statistical analysis

For the eight morphological traits, the effect of the accessions was determined by the analysis of variance (ANOVA). The comparison of the averages was realized by using the test of Student-Newman-Keuls ($\alpha = 0.05$). The statistical analysis also included the elaboration of a matrix of coefficient of simple linear correlation between variables. The statistical analyses were made with the software SAS 9.1 (Anonymous, 1992).

For seed morphology ANOVA was used for the comparison between different groups, post-hoc analysis was carried out using Tukey test (samples of similar sizes) and the analysis was done with SPSS 11.5.

RESULTS

Plant development and seed yield

The number of seedlings surviving varied from 11 (J8) to 24 (J3) (Table 1). After five years, mean plant height oscillated between 178 cm for J4 (Vale do Jequinhonha) and 217 cm for J8 (Tanzanian accession) (Table 1). Canopy circumference (Table 1) oscillated between 368 (J4), and 594 cm, with higher values in the two African accessions (Mozambique, J7 and Tanzania, J8). Plants from Tanzania were higher and had larger canopy circumferences.

Plants from all accessions shed their leaves from December to April; the first leaves and flowers appeared in May. Seed production occurred in three periods: summer, autumn and winter. Seed yield was important in summer and winter, and limited in autumn. Only two Brazilian accessions (Norte de Mina, J1; and Mato-Grosso, J3), were productive in this season with 114 and 151 g of seeds, respectively (Table 2). Seed production was variable in the two other periods. In summer, the highest value was of 256 g (J1), and the lowest of 63 g (J7). In winter, the highest values were 140 g (J8) and the lowest ones of 64 g (J4). In total, Brazilian plants from Paraná (J2) had higher seed yield (467 g), and plants from Mozambique (J7) the lowest (158 g). Yield increased along the years of culture (Table 2).

Positive correlation was found between plant height and canopy circumference ($r^2 = 0.87$), total yield and yield of last harvest (August, 2013; $r^2 = 0.76$), plant

Table 1. Growth parameters: Mean height (cm) and canopy circumference (cm) of each *Jatropha curcas* accession.

Accessions	PH						CC
	June 2008	July 2009	July 2010	July 2011	June 2012	March 2013	June 2012
J1 (n = 22)	48	129	141	152	164	185	483
J2 (n = 21)	38	134	144	157	164	204	474
J3 (n = 24)	50	153	166	170	173	206	408
J4 (n = 14)	32	124	138	152	153	178	368
J5 (n = 22)	46	134	148	161	187	195	481
J6 (n = 22)	54	147	164	176	182	204	548
J7 (n = 16)	44	150	164	178	184	211	586
J8 (n = 11)	25	138	152	170	184	217	594

n: number of plants surviving; PH: plant height; CC: canopy circumference

Table 2. Average of seed yield (g) per plant for each accession. n = number of productive plants

Accessions	December 2011	August 2012	November 2012	January 2013	August 2013	Total
J1	16 (n = 4)	19 (n = 2)	114 (n = 4)	98 (n = 10)	256 (n = 12)	414
J2	35 (n = 6)	112 (n = 3)	0	121 (n = 8)	199 (n = 9)	467
J3	4 (n = 5)	20 (n = 3)	151 (n = 3)	101 (n = 11)	144 (n = 14)	420
J4	7 (n = 1)	15 (n = 15)	0	64 (n = 4)	211 (n = 6)	297
J5	12 (n = 8)	53 (n = 5)	0	112 (n = 8)	215 (n = 10)	393
J6	25 (n = 6)	43 (n = 2)	0	64 (n = 11)	90 (n = 7)	223
J7	7 (n = 5)	8 (n = 4)	0	78 (n = 8)	63 (n = 5)	158
J8	14 (n = 3)	24 (n = 2)	0	140 (n = 7)	171 (n = 8)	350

height and seed yield in winter (0.56), and canopy circumference and seed yield in summer (0.51) and in winter (0.62).

Morphological variability

Analysis of variance revealed high morphological variability with a significant ($p < 0.05$) effect of accession for all five characters under study (FS, LL, LW, PL, NN). The number of groups obtained in ANOVA differs according to the character considered (Table 3). For the parameters of the leaf, J4 forms an isolated group with higher values of LL and PL; and together with J5, both have higher values of LW and FS. All foliar parameters are lower for J7 and J3. The highest number of nodes is observed for J3, and lowest for J4 (Table 3).

Significant ($p < 0.05$) positive correlations were found between all leaf magnitudes (leaf surface (FS), leaf length (LL), leaf width (LW) and petiole length (PL).

Seed morphology

Area and perimeter were lower in seed populations obtained from Suriname (J6) and Mozambique (J7) and higher in seed populations from Tanzania (J8). Length to width ratio was lower in J7 and higher in J3. Correspondingly, circularity index values were lower in J3 and higher in J7. Table 4 summarizes the results obtained for seed morphology.

Seed shapes of *Jatropha curcas* adjust well to an ellipse whose relation between major and minor axes (a and b) equals the Golden Ratio (Fig. 3). The J index oscillates between 0.86 and 0.99 (mean value is 0.95). Mean values are lower in J7 and higher in J1, J3 and J4.

Table 3. Developmental characteristics of plants (FS: Foliar surface area (cm²); LL: Limb length (cm); LW: Limb width (cm); PL: Petiole length (cm); NN: Number of nodes)

Accession	FS	LL	LW	PL	NN
J1	93.20 c,d	10.35 b	11.21 b,c	14.47 b,c	16,12 b
J2	94.18 c,d	10.94 b	11.30 b,c	15.36 b	15.26 b
J3	90.77 c,d	10.26 b	10.75 c	11.70 d	18.15 a
J4	118.83 a	11.86 a	12.43 a	19.08 a	14.97 b
J5	111.15 a,b	11.12 b	11.83 a,b	16.34 b	15.76 b
J6	9.92 c,d	10.46 b	10.86 c	14.62 b,c	15.56 b
J7	87.34 d	10.28 b	10.52 c	12.31 c,d	15.07 b
J8	104.66 b,c	11.82 b	11.43 b,c	16.73 b	16.00 b

Table 4. Morphological characteristics of seeds

Accession	Area	Perimeter	Length/Width ratio	Circularity index	J index
J1	1.40 b,c,d	4.39 b,c	1.60 a,b	0.91 b,c	0.96 b
J2	1.41 c,d	4.41 b,c	1.60 a,b	0.91 a,b	0.95 a,b
J3	1.37 a,b,c,d	4.38 b,c	1.68 c	0.89 a	0.96 b
J4	1.43 c,d	4.45 b,c	1.62 b,c	0.91 a,b	0.96 b
J5	1.36 a,b,c	4.32 a,b	1.60 a,b	0.91 b,c	0.95 a,b
J6	1.27 a	4.18 a	1.60 a,b	0.91 b,c	0.95 a,b
J7	1.30 a,b	4.21 a	1.55 a	0.92 c	0.94 a
J8	1.46 d	4.48 c	1.61 b	0.91 b,c	0.95 a,b

A negative correlation was found, as expected, between length to width ratio and circularity index ($r^2 = -0.97$). Positive correlations were found between length to width ratio and J index ($r^2 = 0.76$).

DISCUSSION

Yield obtained from the eight *Jatropha curcas* accessions used in this study was moderate or low in relationship to other published results as reported in the introduction. This may be due to the high latitude of the experimental site (33°54' N), whereas

the reported limit for cultivation of this species is of 30° N (Anonymous, 2010). A cold winter season with temperature ranges below the optimum may be an important factor limiting seed production. Reduced seed yield may be a consequence of the climatic conditions during seed formation; high temperature and low moisture in summer and cold in winter, temperature being an important factor in growth and productivity of *J. curcas* (Maes et al., 2009). Nevertheless, grain yield increased with age of the plants, as shown by the correlation found between yield of the last harvest (August, 2013) and total yield, and further increases in yield may be expected in successive seasons.

The eight *J. curcas* accessions showed high phenotypic diversity, this result joins that obtained in America (Ovando-Medina et al., 2011b; He, 2011; Brasileiro et al., 2013), China, India and Philippines (Gohil and Pandya, 2009; Vijayanand et al., 2009; Das et al., 2010; Zapico et al., 2011; Guan et al., 2013). However, high morphological variability does not always reflect high genotypic variability. Xu et al. (2012) showed that high phenotypic diversity in most cases was not associated with genotypic diversity, indicating a strong influence for environment. It appears that the environment or particular genotype-environment combinations have predominant roles in the morphological variation among accessions (Ovando-Medina et al., 2011a; Rafii et al., 2012). Our results show high variability in foliar traits, in particular leaf surface. Plant height and seed yield are also different between accessions, this result due probably to genetic-environmental interaction (Heller, 1996). Epigenetics also plays an important role in the variability of *Jatropha* (Yi et al., 2010).

Previous reports using unique varieties present a relation between seed yield and agronomic traits like foliar surface, plant height, number of nodes per branch, and others (Gohil and Pandya, 2009; Rafii et al., 2012; Brasileiro et al., 2013). For example, Sun et al. (2012) identified a total of 28 QTLs (quantitative trait loci) underlying the growth and seed yield traits in *Jatropha* and showed a significant correlation between phenotypic traits and total seed weight. Plant height, stem diameter, branch number, female flower number and fruit number were suggested to be key factors for seed yields. Rafii et al. (2012) showed the important heredity of plant height and indicated that the genetic component was high (> 50%) for seed yield per plant, fruit \times plant⁻¹ and flowering bunches \times plant⁻¹. Brasileiro et al. (2013) used plant height for indirect selection in populations of the physic nut in order to obtain superior genotypes. Our data obtained with plants of eight different accessions grown during five years do not support the existence of general correlations between any of the considered morphological traits and grain yield. Plants in this experiment proceed from diverse origins and thus, genotype-environment interactions are multiple and may result in a lack of general correlation between any particular morphological character of the plants and yield, a

property that may well be observed when considering a unique variety or accession on its own. In addition, the morphological traits were measured in a season previous to the increase in grain yield that took place in the last harvest of August 2013.

The images of *Jatropha curcas* seeds adjust well to an ellipse, whose relation between major and minor axis equals the Golden Ratio. Differences between accessions were found in seed size and shape, showing a trend where larger seeds had also higher values of J index and lower circularity index. Our results suggest a relationship between seed yield, size and shape. Seeds from the accessions J6 and J7 (Suriname and Mozambique respectively), that presented the lowest yield in the last seasons, were smaller and more circular, and their shape adjusted less well to an ellipse (lower J index values). The observed association between reduced yield and seed morphological traits opens the possibility for the development of tests addressed to evaluate the quality of seed lots, based on morphological properties of seeds. Seed shape analysis is simple to perform and may provide an indication on the condition of the seed stocks in relation to yield. This may contribute to germplasm selection for agronomical applications.

The introduction of *J. curcas* is a complex project; the programmes launched in Brazil, Nicaragua and India for introduction of *J. curcas* for varied purposes met with limited success due to the wide gap in potential and actual yields. The species has not been improved for productivity and most of the projects relied on naturally occurring unadapted populations, which are a result of the few initial introductions (Basha and Sujatha, 2007). Thus, *Jatropha curcas* is not common in Mediterranean regions with arid and semi-arid climates; plantations in these regions hold the risk of low productivity or irrigation requirement. Also, plantations in regions with frost risk hold the risk of damage due to frost (Maes et al., 2009). However, the high phenotypic variability and the difference in productivity represented a good potential for early selection. Proposed future research in this species comprises the description of introduced genotypes, the study of the physiological behavior and the improvement of seed yield and oil productivity.

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